Relationship between malondialdehyde level and glutathione peroxidase activity in diabetic rats

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Abstract

Background: This investigation describes the relationship between glutathione peroxidase activities, an antioxidant enzyme, and the oxidative status in diabetic rat blood. Methods: Malondialdehyde level and glutathione peroxidase activity were measured by spectrophotometric techniques. Results and conclusions: Malondialdehyde content in the diabetic rats group was increased compared to that in the controls [3.08 ± 0.32 (mean ± S.E.) vs. 1.15 ± 0.29 mmol/g hemoglobin, \(P>0.01\)]. Glutathione peroxidase activity in the diabetic rats group was increased compared to that in the control [10.27 ± 1.39 (mean ± S.E.) vs. 3.14 ± 0.38 \(\mu\)mol NADPH/min/g hemoglobin, \(P>0.01\)]. Our results show a positive correlation between serum glutathione peroxidase and malondialdehyde concentration in diabetic rats. © 2003 Elsevier B.V. All rights reserved.

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1. Introduction

There is much evidence that reactive oxygen molecules contribute to organ injury in many systems, including the heart, liver, and central nervous system [1–3]. Oxygen free radicals have also been implicated in ischemia–reperfusion renal failure and acute toxin-induced nephropathy [4–6]. Reactive oxygen species (ROS) are constantly formed in the human body and removed by an antioxidant defense system. In healthy individuals, the generation of ROS appears to be approximately in balance with antioxidant defense. An imbalance between ROS and antioxidant defenses in favor of the former has been described as oxidative stress. In some human disease, increased oxidative stress may make an important contribution to disease pathology [7,8]. ROS are generally cytotoxic, because of the oxidative damage they can cause to cellular components. However, at low concentrations, ROS may function as physiological mediators of cellular response. For example, hydrogen peroxide mimics the stimulatory effects of insulin on glucose transport and lipid synthesis in adipocytes [9]. Oxidative stress, which is associated with the formation of lipid peroxides, is suggested to contribute to pathological

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processes in aging and many diseases, such as diabetes, atherosclerosis and cataract [10]. Increased oxidative stress as a result of increased free radical formation has also been suggested as a contributor to vascular damage in diabetes [11–13]. Small amounts of (ROS), including hydroxyl radicals (-OH), superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2), are constantly generated in aerobic organisms in response to both external and internal stimuli [14–17]. In contrast, high doses and/or inadequate removal of ROS result in oxidative stress, which may cause server metabolic malfunction and damage to biological macromolecules [17,18]. Oxidative stress, superoxide production and an imbalance in antioxidant enzymes has been related with many other specific pathologies as chronic granulomatous disease, diabetic complications, hepatitis, rheumatoid arthritis, influenza virus, ulcer, pneumonia, HIV infection, cataract and glaucoma [18,19]. The objective of this study is to illustrate the relationship between an antioxidant enzyme, glutathione peroxidase and oxidative status, malondialdehyde level.

2. Materials and methods

2.1. Materials

EDTA, sodium chloride and thiobarbituric acid were purchased from Sigma (St. Louis, MO, USA). Other reactives such as streptozotocin were purchased from Merck (Germany).

2.2. Animals

All animals survived the study without signs of illness. Male Wistar rats, 10 weeks old and between 220 and 260 g in body weight, were randomly assigned to two groups. One group of rats (diabetic group) received a single tail-vein injection of streptozotocin (50 mg/kg) under light anesthesia with diethyl ether. Streptozotocin was dissolved in a citrate solution (0.15 mol/l citric acid and 0.25 mol/l sodium phosphate, pH 4.6). It takes 1 month for the proposed model to achieve diabetes. Control group received an equivalent volume of 0.15 mol/l citrate buffer alone. Control and diabetic rats were caged separately but housed under similar conditions. Both groups of animals were fed with the same diet and water ad libitum. Blood samples for analyses were collected after 3 months between 7:00 and 8:00 am from each animal. On the day of the experiments, a blood plasma sample was collected and malondialdehyde, glucose level, and glutathione peroxidase activity were determined. The glucose concentration of plasma was measured by Trinder’s method [20].

2.3. Sample

Venous peripheral blood samples (1 ml) from rats were collected in EDTA-coated tubes. Erythrocytes were separated from plasma and mononuclear cells by centrifugation (10 min at 1500 × g) and washed three times with isotonic saline by centrifugation at 1500 × g. After the final wash, the red blood cells were lysed by hypotonic shock using cold distilled water. Different dilution was used as hemolysate.

2.4. Determination of malondialdehyde

Concentration of malondialdehyde was measured by the thiobarbituric acid test [21]. Results were expressed as mmol thiobarbituric acid reactive substances/g hemoglobin.

2.5. Assay of glutathione peroxidase

Glutathione peroxidase activity was determined according to the previously reported method [22]. An enzyme unit was defined as the amount of enzyme that catalyzes the release of μmol NADPH/min at 37 °C. Specific activity was in terms of units per gram hemoglobin. The amount of hemoglobin was determined by Merck test cat. no. 3317.

3. Results

Body weight in diabetic and non-diabetic rats is shown in (Fig. 1). All rats injected with streptozotocin developed severe diabetes as indicated by increasing plasma glucose level (range 228–283 mg/dl). Plasma glucose levels in diabetic rats were elevated approximately threefold as compared with controls. Plasma glucose concentration in diabetic and non-diabetic rats is shown in (Fig. 2). Hemoglobin content in the
diabetic rats group was 1.96 ± 0.03 mmol/l and in the control 2.04 ± 0.04 mol/l. Malondialdehyde content in the diabetic rats group was increased compared to that in the control [3.08 ± 0.32 (mean ± S.E.) vs. 1.15 ± 0.29 mmol/g hemoglobin, P>0.01] (Fig. 3). Glutathione peroxidase activity in the diabetic rats group was increased compared to that in the control [10.27 ± 1.39 (mean ± S.E.) vs. 3.14 ± 0.38 μmol NADPH/min/g hemoglobin, P>0.01] (Fig. 4). Fig. 5 shows relationship between malondialdehyde level and glutathione peroxidase activity in diabetic rats (y=0.61x ± 0.53, r=0.88). There was a positive
correlation between malondialdehyde level and glutathione peroxidase activity.

4. Discussion

As previously demonstrated, reactive oxygen species generated during metabolism can enter into reactions that, when uncontrolled, can affect certain processes leading to clinical manifestations [2,5,7]. Reactive oxygen species are key participants in damage caused by diabetic complications. Oxidative stress, superoxide production and an imbalance in antioxidant enzymes have been related to diabetic complications. Diabetes is one of the pathological processes known to be related with an unbalanced production of reactive oxygen species, such as hydroxyl radicals (HO·), superoxide anions (O₂⁻) and hydrogen peroxide (H₂O₂). Therefore, cells must be protected from this oxidative injury by antioxidant enzymes. Glutathione peroxidase catalyses the reduction of hydroperoxides using glutathione, thereby protecting mammalian cells against oxidative damage. Also, glutathione peroxidase, active in reactive oxygen species neutralization and within cells, removes superoxide and peroxides before they react with metal catalysis to form more reactive species. Determination of malondialdehyde by thiobarbituric acid is used as an index of the extent of lipid peroxidation. As previously demonstrated, malondialdehyde used as the best available measure of global reactive oxygen species was substantially elevated in diabetes [11–13]. In the present study, we found significantly increased glutathione peroxidase activity and malondialdehyde concentrations in diabetic rats as compared with control subjects. Our results confirm previous data of an enhanced reactive oxygen species level in diabetes mellitus [8,11,14]. Higher amounts of reactive oxygen species play a role in the diabetic complications as well as in a number of disease states. As a safeguard against the accumulation of reactive oxygen species, enzymatic antioxidant activities exist. Therefore, when oxidative stress arises as consequence of a pathologic event, a defense system promotes the regulation and expression of antioxidant enzyme such as catalase and glutathione peroxidase. Our results demonstrate that serum glutathione peroxidase activity correlates positively with malondialdehyde level in diabetes. Our findings of the positive relationship of glutathione peroxidase activities and serum malondialdehyde concentrations may support an idea of an imbalance between the antioxidant enzymes system and reactive oxygen species level in diabetes. An overproduction of reactive oxygen species, especially in diabetes, cannot be properly balanced by the antioxidant enzymes.

References